

10/501629

=> d his

(FILE 'HOME' ENTERED AT 11:59:11 ON 18 JAN 2008)

FILE 'MEDLINE, EMBASE, BIOSIS, BIOTECHDS, SCISEARCH, HCAPLUS, NTIS,
LIFESCI' ENTERED AT 11:59:37 ON 18 JAN 2008

L1 23 S AEQUOREA (W)COERULESCENS
L2 17 S (GFP OR FLUORESCENT) AND L1
L3 8833323 S CLON? OR EXPRESS? OR RECOMBINANT
L4 8 S L2 AND (MUTANT OR "222")
L5 3 DUP REM L4 (5 DUPLICATES REMOVED)
 E GURSKAYA N G/AU
L6 79 S E3
 E FRADKOV A F/AU
L7 104 S E3
 E LUKYANOV S A/AU
L8 206 S E3
 E PUNKOVA N I/AU
L9 6 S E3-E6
L10 290 S L6 OR L7 OR L8 OR L9
L11 3 S L1 AND L10
L12 2 DUP REM L11 (1 DUPLICATE REMOVED)

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| | | | |
|--------------|----|-----------------|--|
| NEWS | 1 | | Web Page for STN Seminar Schedule - N. America |
| NEWS | 2 | AUG 06 | CAS REGISTRY enhanced with new experimental property tags |
| NEWS | 3 | AUG 06 | FSTA enhanced with new thesaurus edition |
| NEWS | 4 | AUG 13 | CA/CAplus enhanced with additional kind codes for granted patents |
| NEWS | 5 | AUG 20 | CA/CAplus enhanced with CAS indexing in pre-1907 records |
| NEWS | 6 | AUG 27 | Full-text patent databases enhanced with predefined patent family display formats from INPADOCDB |
| NEWS | 7 | AUG 27 | USPATOLD now available on STN |
| NEWS | 8 | AUG 28 | CAS REGISTRY enhanced with additional experimental spectral property data |
| NEWS | 9 | SEP 07 | STN AnaVist, Version 2.0, now available with Derwent World Patents Index |
| NEWS | 10 | SEP 13 | FORIS renamed to SOFIS |
| NEWS | 11 | SEP 13 | INPADOCDB enhanced with monthly SDI frequency |
| NEWS | 12 | SEP 17 | CA/CAplus enhanced with printed CA page images from 1967-1998 |
| NEWS | 13 | SEP 17 | CAplus coverage extended to include traditional medicine patents |
| NEWS | 14 | SEP 24 | EMBASE, EMBAL, and LEMBASE reloaded with enhancements |
| NEWS | 15 | OCT 02 | CA/CAplus enhanced with pre-1907 records from Chemisches Zentralblatt |
| NEWS | 16 | OCT 19 | BEILSTEIN updated with new compounds |
| NEWS | 17 | NOV 15 | Derwent Indian patent publication number format enhanced |
| NEWS | 18 | NOV 19 | WPIX enhanced with XML display format |
| NEWS | 19 | NOV 30 | ICSD reloaded with enhancements |
| NEWS | 20 | DEC 04 | LINPADOCDB now available on STN |
| NEWS | 21 | DEC 14 | BEILSTEIN pricing structure to change |
| NEWS | 22 | DEC 17 | USPATOLD added to additional database clusters |
| NEWS | 23 | DEC 17 | IMSDRUGCONF removed from database clusters and STN |
| NEWS | 24 | DEC 17 | DGENE now includes more than 10 million sequences |
| NEWS | 25 | DEC 17 | TOXCENTER enhanced with 2008 MeSH vocabulary in MEDLINE segment |
| NEWS | 26 | DEC 17 | MEDLINE and LMEDLINE updated with 2008 MeSH vocabulary |
| NEWS | 27 | DEC 17 | CA/CAplus enhanced with new custom IPC display formats |
| NEWS | 28 | DEC 17 | STN Viewer enhanced with full-text patent content from USPATOLD |
| NEWS | 29 | JAN 02 | STN pricing information for 2008 now available |
| NEWS | 30 | JAN 16 | CAS patent coverage enhanced to include exemplified prophetic substances |
| NEWS EXPRESS | 19 | SEPTEMBER 2007: | CURRENT WINDOWS VERSION IS V8.2, CURRENT MACINTOSH VERSION IS V6.0c(ENG) AND V6.0Jc(JP), AND CURRENT DISCOVER FILE IS DATED 19 SEPTEMBER 2007. |

NEWS EXPRESS 19 SEPTEMBER 2007: CURRENT WINDOWS VERSION IS V8.2,
CURRENT MACINTOSH VERSION IS V6.0c(ENG) AND V6.0Jc(JP),
AND CURRENT DISCOVER FILE IS DATED 19 SEPTEMBER 2007.

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FILE 'HOME' ENTERED AT 11:59:11 ON 18 JAN 2008

=> file medline embase biosis biotechds scisearch hcaplus ntis lifesci
COST IN U.S. DOLLARS SINCE FILE TOTAL
ENTRY SESSION
FULL ESTIMATED COST 0.21 0.21

FILE 'MEDLINE' ENTERED AT 11:59:37 ON 18 JAN 2008

FILE 'EMBASE' ENTERED AT 11:59:37 ON 18 JAN 2008
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FILE 'NTIS' ENTERED AT 11:59:37 ON 18 JAN 2008
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FILE 'LIFESCI' ENTERED AT 11:59:37 ON 18 JAN 2008
COPYRIGHT (C) 2008 Cambridge Scientific Abstracts (CSA)

=> s aequorea (w) coerulescens
L1 23 AEQUOREA (W) COERULESCENS

=> s (GFP or fluorescent) and l1
L2 17 (GFP OR FLUORESCENT) AND L1

```
=> clon? or express? or recombinant  
CLON? IS NOT A RECOGNIZED COMMAND  
The previous command name entered was not recognized by the system.  
For a list of commands available to you in the current file, enter  
"HELP COMMANDS" at an arrow prompt (>).
```

=> s clon? or express? or recombinant
L3 8833323 CLON? OR EXPRESS? OR RECOMBINANT

=> s 12 and (mutant or "222")
I.4.8 I.2 AND (MUTANT OR "222")

=> dup rem 14
PROCESSING COMPLETED FOR L4
L5 3 DUP REM L4 (5 DUPLICATES REMOVED)

=> d 1-3 ibib ab

L5 ANSWER 1 OF 3 BIOTECHDS COPYRIGHT 2008 THE THOMSON CORP. on STN
ACCESSION NUMBER: 2006-19750 BIOTECHDS

TITLE: Novel transgenic zebrafish that expresses Tau, amyloid precursor protein or presenilin polypeptide or their fusion polypeptides, or amyloid beta polypeptide, useful for identifying modulator of Alzheimer's disease;
tau protein, amyloid precursor protein, amyloid-beta or presenilin gene transfer and expression in zebrafish neuron for zebrafish transgenic fish and drug screening

AUTHOR: RUBINSTEIN A L

PATENT ASSIGNEE: ZYGOGEN LLC

PATENT INFO: WO 2006081539 3 Aug 2006

APPLICATION INFO: WO 2006-US3165 27 Jan 2006

PRIORITY INFO: US 2005-647493 27 Jan 2005; US 2005-647493 27 Jan 2005

DOCUMENT TYPE: Patent

LANGUAGE: English

OTHER SOURCE: WPI: 2006-539425 [55]

AB DERWENT ABSTRACT:

NOVELTY - A transgenic zebrafish that expresses (a) a tau polypeptide, amyloid precursor protein (APP), amyloid beta or presenilin polypeptide, comprising a zebrafish neuron specific expression sequence operably linked to a nucleic acid encoding a tau, APP, amyloid beta or presenilin polypeptide, which is expressed in the neurons of the transgenic zebrafish, where the transgenic zebrafish exhibits a pathology associated with Alzheimer's Disease, or (b) a tau, APP or presenilin fusion polypeptide, comprising a zebrafish neuron specific expression sequence operably linked to a nucleic acid encoding a fusion polypeptide comprising a tau, APP or presenilin polypeptide and a fluorescent reporter polypeptide, is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are included for: 1) a transgenic zebrafish that expresses a Tau (fusion) polypeptide; 2) a transgenic zebrafish that expresses an APP (fusion) polypeptide; 3) a transgenic zebrafish that expresses an amyloid beta polypeptide; 4) a transgenic zebrafish that expresses a presenilin (fusion) polypeptide;

BIOTECHNOLOGY - Preferred Zebrafish: The zebrafish further comprises zebrafish neuron specific expression sequence operably linked to a nucleic acid encoding a fluorescent reporter polypeptide e.g. green fluorescent protein (GFP), Aequorea coerulescens green fluorescent protein (AcGFP) and DsRedExpress (DsRed protein). The neuron specific expression sequence is a neuron-specific promoter chosen from an elav promoter and a GATA-2 promoter. The zebrafish neuron specific expression sequence and the sequence encoding the tau, APP, amyloid beta polypeptide are contained in an exogenous construct. The zebrafish develops neurofibrillary tangles, or exhibits neuronal cell damage. The tau, APP polypeptide, amyloid beta or presenilin is a mutant tau, APP, amyloid beta polypeptide or presenilin. The expression sequence comprises an inducible promoter, being an inducible UAS promoter activated by GAL4/VP16. The zebrafish further comprises a nucleic acid encoding a zinc transporter. Preferred Method: Identifying an agent that modulates a pathology associated with disease comprises: a) contacting the zebrafish with a test agent; b) comparing the neuronal pathology of the zebrafish contacted with the test agent to the neuronal pathology of a zebrafish not contacted with the test agent; c) determining the effect of the test agent on the zebrafish, such that if there is a difference in the neuronal pathology of the zebrafish contacted with the test agent and the zebrafish not contacted with the test agent, the test agent is an agent that modulates a

pathology associated with Alzheimer's disease. The difference in neuronal pathology is a decrease in neuronal cell death in the zebrafish contacted with the test agent as compared to the zebrafish not contacted with the test agent or a decrease in neurofibrillary tangles in the zebrafish contacted with the test agent as compared to the zebrafish not contacted with the test agent. The difference in neuronal pathology is a decrease in neuronal fluorescence. The difference in neuronal pathology is a decrease in protein expression in the zebrafish contacted with the test agent as compared to the zebrafish not contacted with the test agent. Identifying an agent that modulates neuronal pathology comprises: a) administering a test agent to a transgenic zebrafish expressing a reporter protein in neurons; b) comparing the expression of the reporter protein in the neurons of the zebrafish contacted with the test agent with the expression of the reporter protein in the neurons of a transgenic zebrafish that was not contacted with the test agent; and c) determining the effect of the test compound on the expression of the reporter protein in the neurons, such that if the number of neurons in the zebrafish contacted with the test agent is greater than the number of neurons in the zebrafish that was not contacted with the test agent, the test agent is an agent that modulates neuronal pathology and is a neuroproliferative agent. The reporter protein is a fluorescent reporter polypeptide.

ACTIVITY - Nootropic; Neuroprotective. No biological data given.

MECHANISM OF ACTION - None given.

USE - For identifying an agent that modulates a pathology associated with Alzheimer's disease (claimed).

ADVANTAGE - The transgenic zebrafish enables identification of an agent that modulates a pathology associated with Alzheimer's disease. (75 pages)

L5 ANSWER 2 OF 3 BIOTECHDS COPYRIGHT 2008 THE THOMSON CORP. on STN

DUPLICATE 1

ACCESSION NUMBER: 2003-22532 BIOTECHDS

TITLE: New nucleic acid molecule present in other than its natural environment and that encodes a fluorescent protein from *Aequorea coerulescens*, useful for various labeling applications;

involving vector-mediated gene transfer and expression in host cell for use in labeling and biosensor

AUTHOR: GURSKAYA N; FRADLOV A; LUKYANOV S; PUNKOVA N

PATENT ASSIGNEE: EVROGEN JSC

PATENT INFO: WO 2003062270 31 Jul 2003

APPLICATION INFO: WO 2003-IB907 17 Jan 2003

PRIORITY INFO: US 2002-351518 22 Jan 2002; US 2002-351518 22 Jan 2002

DOCUMENT TYPE: Patent

LANGUAGE: English

OTHER SOURCE: WPI: 2003-608187 [57]

AB DERWENT ABSTRACT:

NOVELTY - A nucleic acid molecule present in other than its natural environment and that encodes a fluorescent protein from *Aequorea coerulescens*, is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are included for the following: (1) a construct comprising a vector and the above nucleic acid molecule; (2) an expression cassette comprising a transcriptional initiation region functional in an expression host, the above nucleic acid molecule, and a transcriptional termination region functional in the expression host; (3) a cell, or its progeny, comprising the expression cassette; (4) a method of producing a chromo- or fluorescent protein, comprising growing the cell cited above under conditions where the chromo- or fluorescent protein is expressed; (5) a protein or its fragment encoded by the above nucleic acid, or a protein or its fragment having a sequence similarity of at least about 95% to the above-mentioned protein or fragment; (6) a fusion protein incorporating the protein or fragment cited above; (7) an antibody binding specifically

to the above protein; (8) a transgenic organism comprising the above nucleic acid; and (9) a kit comprising the above nucleic acid and instructions for using the nucleic acid.

BIOTECHNOLOGY - Preferred Nucleic Acid: The nucleic acid is isolated. It encodes a fluorescent protein comprising any of the 12 amino acid sequences not clearly defined in the specification. The nucleic acid comprises a sequence that is substantially similar to or identical to a nucleotide sequence of at least 10 residues in length taken from any of the 12 nucleotide sequences not clearly defined in the specification. Alternatively, the nucleic acid has a sequence similarity of at least about 70% with any of the above-mentioned nucleotide sequences. Additionally, the nucleic acid encodes a mutant fluorescent protein comprising at least one point mutation or at least one deletion mutation as compared to a wild-type protein. The nucleic acid or its mimetic may hybridize under stringent conditions to a similar nucleic acid or its complements or fragments. **Preferred Method:** Producing a chromo- or fluorescent protein further comprises isolating the chromo- or fluorescent protein substantially free of other proteins. **Preparation:** The nucleic acid molecule was prepared using standard isolation techniques.

USE - The nucleic acid molecule and protein are useful in labeling applications, in fluorescence resonance energy transfer methods, or as biosensors in prokaryotic and eukaryotic cells. (76 pages)

| | | |
|-------------------|---|-------------|
| L5 ANSWER 3 OF 3 | MEDLINE on STN | DUPLICATE 2 |
| ACCESSION NUMBER: | 2003313870 MEDLINE | |
| DOCUMENT NUMBER: | PubMed ID: 12693991 | |
| TITLE: | A colourless green fluorescent protein homologue from the non-fluorescent hydromedusa Aequorea coerulescens and its fluorescent mutants. | |
| AUTHOR: | Gurskaya Nadya G; Fradkov Arkady F; Pounkova Natalia I; Staroverov Dmitry B; Bulina Maria E; Yanushevich Yurii G; Labas Yulii A; Lukyanov Sergey; Lukyanov Konstantin A Shemyakin and Ovchinnikov Institute of Bioorganic Chemistry RAS, Miklukho-Maklaya 16/10, Moscow 117997, Russia. | |
| CORPORATE SOURCE: | | |
| SOURCE: | The Biochemical journal, (2003 Jul 15) Vol. 373, No. Pt 2, pp. 403-8.
Journal code: 2984726R. ISSN: 0264-6021. | |
| PUB. COUNTRY: | England: United Kingdom | |
| DOCUMENT TYPE: | (COMPARATIVE STUDY)
Journal; Article; (JOURNAL ARTICLE)
(RESEARCH SUPPORT, NON-U.S. GOV'T) | |
| LANGUAGE: | English | |
| FILE SEGMENT: | Priority Journals | |
| OTHER SOURCE: | GENBANK-AY151052; GENBANK-AY233272 | |
| ENTRY MONTH: | 200308 | |
| ENTRY DATE: | Entered STN: 8 Jul 2003
Last Updated on STN: 16 Aug 2003
Entered Medline: 15 Aug 2003 | |

AB We have cloned an unusual colourless green fluorescent protein (GFP)-like protein from *Aequorea coerulescens* (acGFPL). The *A. coerulescens* specimens displayed blue (not green) luminescence, and no fluorescence was detected in these medusae. *Escherichia coli* expressing wild-type acGFPL showed neither fluorescence nor visible coloration. Random mutagenesis generated green fluorescent mutants of acGFPL, with the strongest emitters found to contain an Glu(222) --> Gly (E222G) substitution, which removed the evolutionarily invariant Glu(222). Re-introduction of Glu(222) into the most fluorescent random mutant, named aceGFP, converted it into a colourless protein. This colourless aceGFP-G222E protein demonstrated a novel type of UV-induced photoconversion, from an immature non-fluorescent form into a green fluorescent form.

Fluorescent aceGFP may be a useful biological tool, as it was able to be expressed in a number of mammalian cell lines. Furthermore, expression of a fusion protein of 'humanized' aceGFP and beta-actin produced a fluorescent pattern consistent with actin distribution in mammalian cells.

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L1 23 S AEQUOREA (W)COERULESCENS
L2 17 S (GFP OR FLUORESCENT) AND L1
L3 8833323 S CLON? OR EXPRESS? OR RECOMBINANT
L4 8 S L2 AND (MUTANT OR "222")
L5 3 DUP REM L4 (5 DUPLICATES REMOVED)

=> e Gurskaya n g/au

E1 1 GURSKAYA MARINA/AU
E2 12 GURSKAYA N/AU
E3 79 --> GURSKAYA N G/AU
E4 10 GURSKAYA N I/AU
E5 10 GURSKAYA N V/AU
E6 41 GURSKAYA N Z/AU
E7 2 GURSKAYA NADEJDA/AU
E8 3 GURSKAYA NADEZDA GEORGIEVNA/AU
E9 3 GURSKAYA NADIA/AU
E10 2 GURSKAYA NADIA G/AU
E11 3 GURSKAYA NADYA/AU
E12 39 GURSKAYA NADYA G/AU

=> s e3

L6 79 "GURSKAYA N G"/AU

=> e fradkov a f/au

E1 2 FRADKOV A A/AU
E2 39 FRADKOV A B/AU
E3 104 --> FRADKOV A F/AU
E4 5 FRADKOV A I/AU
E5 112 FRADKOV A L/AU
E6 9 FRADKOV A S/AU
E7 3 FRADKOV ALEXANDER/AU
E8 6 FRADKOV ALEXANDER L/AU
E9 6 FRADKOV ARCADY/AU
E10 2 FRADKOV ARCADY F/AU
E11 15 FRADKOV ARCADY FEDOROVICH/AU
E12 9 FRADKOV ARKADY/AU

=> s e3

L7 104 "FRADKOV A F"/AU

=> e lukyanov s a/au

E1 1 LUKYANOV RS/AU
E2 244 LUKYANOV S/AU
E3 206 --> LUKYANOV S A */AU
E4 2 LUKYANOV S A */AU
E5 1 LUKYANOV S G/AU
E6 18 LUKYANOV S I/AU
E7 19 LUKYANOV S L/AU
E8 269 LUKYANOV S M/AU
E9 3 LUKYANOV S N/AU
E10 4 LUKYANOV S P/AU

E11 1 LUKYANOV S S/AU
E12 26 LUKYANOV S V/AU

=> s e3
L8 206 "LUKYANOV S A"/AU

=> e punkova n i/au
E1 1 PUNKOVA N/AU
E2 1 PUNKOVA N G/AU
E3 1 --> PUNKOVA N I/AU
E4 1 PUNKOVA N L/AU
E5 1 PUNKOVA NATALIA/AU
E6 3 PUNKOVA NATALIA I/AU
E7 1 PUNKOVIC N/AU
E8 1 PUNKOVSKII A N/AU
E9 8 PUNKRUT W/AU
E10 43 PUNKT J/AU
E11 7 PUNKT JUERGEN/AU
E12 99 PUNKT K/AU

=> s e3-e6
L9 6 ("PUNKOVA N I"/AU OR "PUNKOVA N L"/AU OR "PUNKOVA NATALIA"/AU
OR "PUNKOVA NATALIA I"/AU)

=> d his

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LIFESCI' ENTERED AT 11:59:37 ON 18 JAN 2008

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L2 17 S (GFP OR FLUORESCENT) AND L1
L3 8833323 S CLON? OR EXPRESS? OR RECOMBINANT
L4 8 S L2 AND (MUTANT OR "222")
L5 3 DUP REM L4 (5 DUPLICATES REMOVED)
E GURSKAYA N G/AU
L6 79 S E3
E FRADKOV A F/AU
L7 104 S E3
E LUKYANOV S A/AU
L8 206 S E3
E PUNKOVA N I/AU
L9 6 S E3-E6

=> s l6 or l7 or l8 or l9
L10 290 L6 OR L7 OR L8 OR L9

=> s l1 and l10
L11 3 L1 AND L10

=> dup rem l11
PROCESSING COMPLETED FOR L11
L12 2 DUP REM L11 (1 DUPLICATE REMOVED)

=> d 1-2 ibib ab

L12 ANSWER 1 OF 2 HCAPLUS COPYRIGHT 2008 ACS on STN
ACCESSION NUMBER: 2003:591209 HCAPLUS
DOCUMENT NUMBER: 139:129175
TITLE: Sequences of novel fluorescent proteins from Aequorea
coeruleus and use
INVENTOR(S): Gurskaya, Nadejda; Fradlov, Arkadiy; Lukyanov, Sergey;
Punkova, Natalia
PATENT ASSIGNEE(S): Evrogen, Jsc, USA

SOURCE: PCT Int. Appl., 76 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

| PATENT NO. | KIND | DATE | APPLICATION NO. | DATE |
|------------------------|---|----------|-----------------|------------|
| WO 2003062270 | A2 | 20030731 | WO 2003-IB907 | 20030117 |
| WO 2003062270 | A3 | 20031127 | | |
| WO 2003062270 | B1 | 20040401 | | |
| WO 2003062270 | A8 | 20041104 | | |
| W: | AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN,
CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH,
GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR,
LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH,
PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, TJ, TM, TN, TR, TT, TZ,
UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW | | | |
| RW: | GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AM, AZ, BY,
KG, KZ, MD, RU, TJ, TM, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES,
FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, SE, SI, SK, TR, BF,
BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG | | | |
| CA 2474108 | A1 | 20030731 | CA 2003-2474108 | 20030117 |
| EP 1485481 | A2 | 20041215 | EP 2003-706812 | 20030117 |
| R: | AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT,
IE, SI, LT, LV, FI, RO, MK, CY, AL, TR, BG, CZ, EE, HU, SK | | | |
| JP 2005526495 | T | 20050908 | JP 2003-562147 | 20030117 |
| US 2006167225 | A1 | 20060727 | US 2004-501629 | 20040715 |
| PRIORITY APPLN. INFO.: | | | US 2002-351518P | P 20020122 |
| | | | WO 2003-IB907 | W 20030117 |

AB The present invention provides protein and cDNA sequences of a novel colorless GFP-like protein, acGFP, from *Aequorea coerulscens* and fluorescent and non-fluorescent mutants and derivs. thereof, as well as peptides and proteins encoded by these nucleic acid compns. The subject protein and nucleic acid compns. of the present invention are colored and/or fluorescent and/or can be photoactivated, and can be used in a variety of different biol. applications, particularly for labeling. Finally, kits for use in such biol. applications are provided.

L12 ANSWER 2 OF 2 EMBASE COPYRIGHT (c) 2008 Elsevier B.V. All rights reserved on STN DUPLICATE 1

ACCESSION NUMBER: 2003300328 EMBASE
TITLE: A colourless green fluorescent protein homologue from the
non-fluorescent hydromedusa *Aequorea*
coeruleescens and its fluorescent mutants.
AUTHOR: Gurskaya N.G.; Fradkov A.F.; Pounkova
N.I.; Staroverov D.B.; Bulina M.E.; Yanushevich Y.G.; Labas
Y.A.; Lukyanov S.; Lukyanov K.A.
CORPORATE SOURCE: K.A. Lukyanov, Shemyakin/Ovchinnikov Inst. B.,
Miklukho-Maklaya 16/10, Moscow 117997, Russian Federation.

kluk@ibch.ru

SOURCE: Biochemical Journal, (15 Jul 2003) Vol. 373, No. 2, pp.

403-408.

Refs: 25

ISSN: 0264-6021 CODEN: BIJOAK

COUNTRY: United Kingdom

DOCUMENT TYPE: Journal; Article

FILE SEGMENT: 029 Clinical and Experimental Biochemistry

LANGUAGE: English

SUMMARY LANGUAGE: English

ENTRY DATE: Entered STN: 14 Aug 2003

Last Updated on STN: 14 Aug 2003

AB We have cloned an unusual colourless green fluorescent protein (GFP)-1

protein from *Aequorea coerulescens* (acGFP). The *A. coerulescens* specimens displayed blue (not green) luminescence, and no fluorescence was detected in these medusae. *Escherichia coli* expressing wild-type acGFP showed neither fluorescence nor visible coloration. Random mutagenesis generated green fluorescent mutants of acGFP, with the strongest emitters found to contain an Glu(222) → Gly (E222G) substitution, which removed the evolutionarily invariant Glu(222). Reintroduction of Glu(222) into the most fluorescent random mutant, named aceGFP, converted it into a colourless protein. This colourless aceGFP-G222E protein demonstrated a novel type of UV-induced photoconversion, from an immature non-fluorescent form into a green fluorescent form. Fluorescent aceGFP may be a useful biological tool, as it was able to be expressed in a number of mammalian cell lines. Furthermore, expression of a fusion protein of 'humanized' aceGFP and β-actin produced a fluorescent pattern consistent with actin distribution in mammalian cells.

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L8 206 S E3
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L9 6 S E3-E6.
L10 290 S L6 OR L7 OR L8 OR L9
L11 3 S L1 AND L10
L12 2 DUP REM L11 (1 DUPLICATE REMOVED)

| | Document ID | Kind Codes | Source | Issue Date | Pages |
|---|----------------------|------------|--------------|------------|-------|
| 1 | US 20070298412
A1 | | US-
PGPUB | 20071227 | 38 |
| 2 | US 20060167225
A1 | | US-
PGPUB | 20060727 | 56 |

| | Title |
|---|---|
| 1 | Fluorescent Proteins And Chromoproteins From Non-Aequorea Hydrozoa Species And Methods For Using Same |
| 2 | Novel fluorescent protein from aequorea coerulscens and methods for using the same |

EAST Search History

| Ref # | Hits | Search Query | DBs | Default Operator | Plurals | Time Stamp |
|-------|------|---------------------------|--------------------|------------------|---------|------------------|
| L1 | 12 | aequorea adj coerulescens | US-PGPUB;
USPAT | OR | OFF | 2008/01/18 12:11 |
| L2 | 2 | (mutant or "222") same l1 | US-PGPUB;
USPAT | OR | OFF | 2008/01/18 12:10 |
| L3 | 337 | LUKYANOV FRADKOV GURSKAYA | US-PGPUB;
USPAT | OR | OFF | 2008/01/18 12:18 |
| L4 | 2 | l1 and l3 | US-PGPUB;
USPAT | OR | OFF | 2008/01/18 12:18 |

| | Document ID | Kind Codes | Source | Issue Date | Pages |
|----|----------------------|-------------------|---------------|-------------------|--------------|
| 1 | US 20070298412
A1 | | US-
PGPUB | 20071227 | 38 |
| 2 | US 20070266458
A1 | | US-
PGPUB | 20071115 | 77 |
| 3 | US 20070072267
A1 | | US-
PGPUB | 20070329 | 26 |
| 4 | US 20070015229
A1 | | US-
PGPUB | 20070118 | 29 |
| 5 | US 20060257886
A1 | | US-
PGPUB | 20061116 | 12 |
| 6 | US 20060188890
A1 | | US-
PGPUB | 20060824 | 21 |
| 7 | US 20060167225
A1 | | US-
PGPUB | 20060727 | 56 |
| 8 | US 20050181453
A1 | | US-
PGPUB | 20050818 | 32 |
| 9 | US 20050032132
A1 | | US-
PGPUB | 20050210 | 24 |
| 10 | US 20040248208
A1 | | US-
PGPUB | 20041209 | 72 |
| 11 | US 20040171067
A1 | | US-
PGPUB | 20040902 | 89 |
| 12 | US 20040043490
A1 | | US-
PGPUB | 20040304 | 17 |

| | Document ID | Kind Codes | Source | Issue Date | Pages |
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| 1 | US 20060167225
A1 | | US-
PGPUB | 20060727 | 56 |
| 2 | US 20050032132
A1 | | US-
PGPUB | 20050210 | 24 |

| | Title |
|---|--|
| 1 | Novel fluorescent protein from
aequorea coerulscens and
methods for using the same |
| 2 | Cancer diagnostics |

Attachment #4

GenCore version 6.2.1
Copyright (c) 1993 - 2008 Biocceleration Ltd.

OM protein - protein search, using sw model

Run on: January 18, 2008, 11:27:26 ; Search time 1 Seconds
(without alignments)
0.057 Million cell updates/sec

Title: US-10-501-629-2

Perfect score: 1209

Sequence: 1 MSKGAELFTGVVPILIELNG.....IYFEFVTAAAITHGMDELYK 238

Scoring table: PAM320

Gapop 1.0 , Gapext 0.1

Scoring matrix -Pam320

gap penalty -1

1 gap size penalty -0.1

Searched: 1 seqs, 238 residues

Total number of hits satisfying chosen parameters:

Minimum DB seq length: 0

Maximum DB seq length: 2000000000

Post-processing: Minimum Match 0%

Maximum Match 100%

Listing first 45 summaries

Database : 6919186.pep:*

Pred. No. is the number of results predicted by chance to have a score greater than or equal to the score of the result being printed, and is derived by analysis of the total score distribution.

SUMMARIES

%

| Result No. | Query Score | Match Length | DB ID | Description |
|------------|-------------|--------------|-------|-----------------------------------|
| 1 | 1165 | 96.4 | 238 1 | US-09-967-301-3 Sequence 3, Appli |

ALIGNMENTS

RESULT 1

US-09-967-301-3

; Sequence 3, Application US/09967301

; Patent No. 6919186

; GENERAL INFORMATION:

; APPLICANT: Stubbs, Simon L.

; APPLICANT: Jones, Anne E.

; APPLICANT: Michael, Nigel P.

; APPLICANT: Thomas, Nicholas

; TITLE OF INVENTION: Fluorescent Proteins

; FILE REFERENCE: PA0111

; CURRENT APPLICATION NUMBER: US/09/967,301
; CURRENT FILING DATE: 2001-09-28
; PRIOR APPLICATION NUMBER: GB 0109858.1
; PRIOR FILING DATE: 2001-04-23
; NUMBER OF SEQ ID NOS: 19
; SOFTWARE: PatentIn Ver. 2.1
; SEQ ID NO 3
; LENGTH: 238
; TYPE: PRT
; ORGANISM: Artificial Sequence
; FEATURE:
; OTHER INFORMATION: Description of Artificial Sequence: synthetic
; OTHER INFORMATION: protein

US-09-967-301-3

Query Match 96.4%; Score 1165; DB 1; Length 238;
Best Local Similarity 91.2%; Pred. No. 0;
Matches 217; Conservative 16; Mismatches 5; Indels 0; Gaps 0;

| | | |
|----|--|-----|
| Qy | 1 MSKGAEELFTGVVPILIELNGDVNGHKFSVSGECEGEGDATYKLTLKFICTTGKLPVPWPTL | 60 |
| | : : | |
| Db | 1 MSKGEEELFTGVVPILVELGDVNGHKFSVSGECEGEGDATYKLTLKFICTTGKLPVPWPTL | 60 |
| Qy | 61 VTTFSYGVQCFSRYPDHMKQHDFFKSAMPEGYIQERTIFKDDGNYKSRAEVKFEGDTLV | 120 |
| | : : : : : | |
| Db | 61 VTTLSYGVQCFSRYPDHMKRHDFFKSAMPEGYVQERTIFKDDGNYKTRAEVKFEGDTLV | 120 |
| Qy | 121 NRIELTGTDFKEDGNILGNKMEYNNAHNVYIMTDKAKNGIKVNFKIRHNIEDGSVQLAD | 180 |
| | : : : : | |
| Db | 121 NRIELKGIDFKEDGNILGHKLEYNNNSHNVYIMADKQKNGIKVNFKIRHNIEDGGVQLAD | 180 |
| Qy | 181 HYQQNTPIGDGPVLLPDNHYLSTQSTSLSKDPNEKRDHMIYFEFVTAAITHGMDELYK | 238 |
| | : : : : | |
| Db | 181 HYQQNTPIGDGPVLLPDNHYLSTSALSLSKDPNEKRDHMVLLGFVTAAGITHGMDELYK | 238 |

Search completed: January 18, 2008, 11:27:26
Job time : 1 secs